REMARKS

Please replace the amendments to page 2, line 31 - page 119, Table 6 submitted on pages 3 and 4 in the Preliminary Amendment filed with the Office on December 29, 2000 (co-filed with the above-referenced application) with the specification amendments submitted herein. It has come to our attention that the page and line numbers quoted in the December 29, 2000 Preliminary Amendment contain inadvertent errors and therefore we request the previously-filed specification amendments be replaced with those listed herein in order to avoid confusion due to the misquotation of page and line numbers. The amendments to the specification listed herein are not intended to replace the amendments to the title, cross-reference to related applications or claims submitted in the Preliminary Amendment filed December 29, 2000.

Amendment of the specification corrects minor typographical errors and as such does not constitute new matter. In the specification on page 5, a typographical error in the structure of the sulfone, S(=O)2, functionality has been corrected. Support for the amendment appears on page 5, line 18 and lines 25-26, which recite an "α,β-unsaturated sulfone". A sulfone group, as is known in the art, includes two oxygen atoms, and comprises the structure -S(=O)2-. The amendmends to pages to replace the term "chemical" with the term "synthetic" are made in order to more particularly point out that, while all molecules are "chemical", those intended for use in the present invention are synthetic. Support for this amendment is found throughout the specification, see, for example, Example 5.

Support for additional amendment of the specification and Figure 13 to correct typographical errors can be found throughout the specification and, for example, on page 6, page 30, page 35, page 40, page 50, page 54, page 62, page 68, page 70, page 74, page 85, page 98, page 100, page 117, page 118 and Figure 15. No new matter is believed to have been added.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "**Version with markings to show changes made**", with deletions shown as strikethrough and insertions shown as double underline. Single underlines in the text are as appears in the specification as filed.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, Applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952 referencing docket number 252312005706**. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: May 31, 2002

Respectfully submitted,

By: _____

Madeline I. Johnston
Registration No. 36,174

Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, California 94304-1018
Telephone: (650) 813-5840
Facsimile: (650) 494-0792

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Replacement paragraph for paragraph beginning on page 2, line 30, and continuing on page 3, through line 2:

--Other references describe conjugates of nonimmunogenic polymers and immunogens (~~Saski~~ Sasaki et al., Scand. J. Immun. (1982) 16:191-200; Sehon, Prog. Allergy (1982) 32:161-202; Wilkinson et al., J. Immunol. ~~(987)~~ (1987) 139:326-331, and Borel et al., J. Immunol. Methods (1990) 126:159-168).--

Replacement paragraph for paragraph on page 3 beginning at line 9:

--In sum, applicants believe that the prior art shows only ill-defined chemical compounds or compounds with numerous non-specific attachment sites employed as valency platform molecules in conjugates. Because the valency of such compounds, the specific location of the attachment sites, and the number of attachment sites are unpredictable and ~~fluctuates~~ fluctuate widely, prior art conjugates comprising such compounds cannot be made reproducibly and show wide ranges in their reported activity.--

Replacement paragraph for paragraph on page 3 beginning at line 20:

In contrast to the above-described art, applicants have developed conjugates comprising chemically-defined, non-polymeric valency platform molecules wherein the valency of the platform molecules is predetermined and wherein each attachment site is available for binding of a biological or chemical molecule. Valency platform molecules within the present invention are defined with respect to their ~~chemical~~ synthetic structure, valency, homogeneity and a defined chemistry which is amenable to effective conjugation with the appropriate biological and/or ~~chemical~~ synthetic molecules.--

Replacement paragraph for paragraph on page 4 beginning at line 29 and continuing through page 5, line 2:

--more preferably, $G^{[2]}$, if present, is a radical derived from a ~~polyalcohol~~ polyether, a polyamine, or a polyglycol; most preferably, $G^{[2]}$ is selected from the group $-(CH_2)_q-$ wherein q=0 to 20, $-CH_2(CH_2OCH_2)_rCH_2-$, wherein r=0 to 300, and ~~$C(CH_2OCH_2CH_2-)_s(OH)_{4-s}$~~ $C(CH_2OCH_2CH_2-)_s(CH_2OH)_{4-s}$ wherein s=1 to 4, more preferably s=3 to 4;--

Replacement paragraph for paragraph on page 5 beginning at line 3:

--each of the $n^{[1]}$ moieties shown as $T^{[1]}$ and each of the $p^{[2]} \times n^{[2]}$ moieties shown as $T^{[2]}$ is independently chosen from the group NHR^{SUB} (amine), $C(=O)NHNHR^{SUB}$ (hydrazide), $NHNHR^{SUB}$ (hydrazine), C(=O)OH (carboxylic acid), $C(=O)OR^{ESTER}$ (activated ester), $C(=O)OC(=O)R^B$ (anhydride), C(=O)X (acid halide), $S(=O)_2X$ (sulfonyl halide), $C(=NR^{SUB})OR^{SUB}$ (imidate ester), NCO (isocyanate), NCS (isothiocyanate), OC(=O)X (haloformate), $C(=O)OC(=NR^{SUB})NHR^{SUB}$ (carbodiimide adduct), C(=O)H (aldehyde), $C(=O)R^B$ (ketone), SH (sulfhydryl or thiol), OH (alcohol), $C(=O)CH_2X$ (haloacetyl), $R^{ALK}X$ (alkyl halide), $S(=O)_2OR^{ALK}X$ (alkyl sulfonate), NR^1R^2 wherein R^1R^2 is -C(=O)CH=CHC(=O)- (maleimide), $C(=O)CR^B=CR^B_2$ (α,β-unsaturated carbonyl), R^{ALK}-Hg-X (alkyl mercurial), and ~~$S(=O)CR^B=CR^B_2$~~ $S(=O)_2CR^B=CR^B_2$ (α,β-unsaturated sulfone);--

Replacement paragraph for paragraph on page 5 beginning at line 19:

--more preferably each of the $n^{[1]}$ moieties shown as $T^{[1]}$ and each of the $p^{[2]} \times n^{[2]}$ moieties shown as $T^{[2]}$ is independently chosen from the group NHR^{SUB} (amine), $C(=O)CH_2X$ (haloacetyl), $R^{ALK}X$ (alkyl halide), $S(=O)_2OR^{ALK}X$ (alkyl sulfonate), NR^1R^2 wherein R^1R^2 is ~~C(=O)CHCHC(=O)~~ -C(=O)CH=CHC(=O)- (maleimide), $C(=O)CR^B=CR^B_2$ (α,β-unsaturated carbonyl), R^{ALK}-Hg-X (alkyl mercurial), and ~~$S(=O)CR^B=CR^B_2$~~ $S(=O)_2CR^B=CR^B_2$ (α,β-unsaturated sulfone);--

Replacement paragraph for paragraph on page 5, beginning at line 27:

--even more preferably each of the $n^{[1]}$ moieties shown as $T^{[1]}$ and each of the $p^{[2]} \times n^{[2]}$ moieties shown as $T^{[2]}$ is independently chosen from the group NHR^{SUB} (amine), $C(=O)CH_2X$ (haloacetyl), NR^1R^2 wherein R^1R^2 is ~~-C(=O)CHCHC(=O)-~~ -C(=O)CH=CHC(=O)- (maleimide), and $C(=O)CR^B=CR^B_2$ (α,β-unsaturated carbonyl);--

Replacement paragraph for paragraph on page 6, beginning at line 15:

--each R^{ESTER} is independently N-succinimidyl, p-nitrophenyl, pentafluorophenyl, tetrafluorophenyl, pentachlorophenyl, 2,4,5-trichlorophenyl, 2,4-dinitrophenyl, cyanomethyl and the like, or other activating group such as ~~5-chloro,8-quinolone, 1-piperidine, N-benzotriazole~~ 5-chloro-8-quinolon-1-yl, 1-piperidyl, 1-benzotriazolyl and the like;--

Replacement paragraph for paragraph beginning on page 9, line 27 continuing through page 10, line 9:

--Exemplary of suitable linker molecules within the present invention are 6 carbon thiols such as HAD, a thio-6 carbon chain phosphate, and HAD_pS, a thio-6 carbon chain phosphorothioate. Chemically-defined valency platform molecules within the present invention are formed, for example, by reacting amino modified-PEG with 3,5-bis-(iodoacetamido) benzoyl chloride (hereinafter ~~"DABA"~~ "IA DABA"); 3-carboxypropionamide-N,N-bis-[(6'-N'-carbobenzyloxyaminohexyl)acetamide] 4"-nitrophenyl ester (hereinafter "BAHA"); 3-carboxypropionamide-N,N-bis-[(8'-N'-carbobenzyloxyamino-3',6'-dioxaoctyl)acetamide] 4"-nitrophenyl ester (hereinafter "$BAHA_{ox}$"); or by reacting PEG-bis-chloroformate with N,N-di(2-[6'-N'-carbobenzyloxyaminohexanoamido]ethyl)amine (hereinafter "AHAB") to form chemically-defined valency platform molecules.--

Replacement paragraph for paragraph on page 10 beginning at line 10:

--Surprisingly unexpected results of at least approximately ten fold up to more than one-hundred fold increase in immunosuppression are achieved using conjugates comprising the chemically-defined, non-polymeric valency platform molecules of the instant invention and biological or ~~chemical~~ synthetic molecules (non-haptens) when compared to the polymeric carriers described in the prior art. For example, at least a one hundred-fold increase in the immunosuppression of anti-dsDNA autoantibodies was achieved as described herein using conjugates within the present invention comprising chemically-defined, non-polymeric valency platform molecules when compared to conjugates comprising an ill-defined carrier described in the prior art.--

Replacement paragraph for paragraph on page 12 beginning at line 12:

--each of $G^{[6]}$ and $G^{[7]}$, if present, is independently a linear, branched or multiply-branched chain comprising 1-2000, more preferably 1-1000, chain atoms selected from the group C, N, O, Si, P and S; more preferably, each of $G^{[6]}$ and $G^{[7]}$ is a radical derived from a polyalcohol, a polyamine, or a polyglycol; most preferably, each of $G^{[6]}$ and $G^{[7]}$ is selected from the group $-(CH_2)_q-$ wherein q=0 to 20, $-CH_2(CH_2OCH_2)_rCH_2-$, wherein r=0 to 300, and ~~$C(CH_2OCH_2CH_2-)_s(OH)_{4-s}$~~ $C(CH_2OCH_2CH_2-)_s(CH_2OH)_{4-s}$ wherein s=1 to 4, more preferably s=3 to 4;--

Replacement paragraph for paragraph on page 12 beginning at line 22 continuing through page 13, line 3:

--each of the $n^{[6]} \times p^{[6]}$ moieties shown as $T^{[6]}$ and each of the $n^{[7]} \times p^{[7]}$ moieties shown as $T^{[7]}$ is independently chosen from the group NHR^{SUB} (amine), $C(=O)NHNHR^{SUB}$ (hydrazide), $NHNHR^{SUB}$ (hydrazine), C(=O)OH (carboxylic acid), $C(=O)OR^{ESTER}$ (activated ester), $C(=O)OC(=O)R^{B}$ (anhydride), C(=O)X (acid halide), $S(=O)_2X$ (sulfonyl halide),

$C(=NR^{SUB})OR^{SUB}$ (imidate ester), NCO (isocyanate), NCS (isothiocyanate), OC(=O)X (haloformate), $C(=O)OC(=NR^{SUB})NHR^{SUB}$ (carbodiimide adduct), C(=O)H (aldehyde), $C(=O)R^{B}$ (ketone), SH (sulfhydryl or thiol), OH (alcohol), $C(=O)CH_2X$ (haloacetyl), $R^{ALK}X$ (alkyl halide), $S(=O)_2OR^{ALK}X$ (alkyl sulfonate), NR^1R^2 wherein R^1R^2 is ~~-C(=O)CHCHC(=O)-~~ -C(=O)CH=CHC(=O)- (maleimide), $C(=O)CR^{B}=CR^{B}_2$ (α,β-unsaturated carbonyl), R^{ALK}-Hg-X (alkyl mercurial), and ~~$S(=O)CR^{B}=CR^{B}_2$~~ $S(=O)_2CR^{B}=CR^{B}_2$ (α,β-unsaturated sulfone);--

Replacement paragraph for paragraph on page 13, beginning at line 4:

--more preferably, each of the $n^{[6]} \times p^{[6]}$ moieties shown as $T^{[6]}$ and each of the $n^{[7]} \times p^{[7]}$ moieties shown as $T^{[7]}$ is independently chosen from the group NHR^{SUB} (amine), $C(=O)CH_2X$ (haloacetyl), $R^{ALK}X$ (alkyl halide), $S(=O)_2OR^{ALK}X$ (alkyl sulfonate), NR^1R^2 wherein R^1R^2 is ~~-C(=O)CHCHC(=O)-~~ -C(=O)CH=CHC(=O)- (maleimide), $C(=O)CR^{B}=CR^{B}_2$ (α,β-unsaturated carbonyl), R^{ALK}-Hg-X (alkyl mercurial), and ~~$S(=O)CR^{B}=CR^{B}_2$~~ $S(=O)_2CR^{B}=CR^{B}_2$ (α,β-unsaturated sulfone);--

Replacement paragraph for paragraph on page 13 beginning at line 27:

--each R^{SUB} is independently H, linear, branched, or cyclic alkyl (1-20C), aryl ~~(1-20C)~~ (6-20C), or alkaryl ~~(1-30C)~~ (7-30C);--

Replacement paragraph for paragraph on page 13 beginning at line 29:

--each R^{ESTER} is independently ~~N-hydroxysuccinimidyl, p-nitrophenoxy, pentafluorophenoxy~~ N-succinimidyl, p-nitrophenyl, pentafluorophenyl, tetrafluorophenyl, pentachlorophenyl, 2,4,5-trichlorophenyl, 2,4-dinitrophenyl, cyanomethyl and the like, or other activating group;--

Replacement paragraph for paragraph on page 15 beginning at line 9:

--Figure 1 shows the anti-PN response in mice primed with PN-KLH, treated with [$(PN)_{20}$-BAHA]-EDDA, Conjugate 17-II, in the doses shown or with saline, which were given a booster injection of PN-KLH and then bled 5 days later. Sera were tested at 3 dilutions by the Farr assay using radiolabeled PN at 10^{-8} M and the data are presented as the percentage reduction of anti-PN antibodies. There were 5 mice per group.--

Replacement paragraph for paragraph on page 16, beginning on line 13:

--Figures ~~6A-B~~ 6A-C show the structure of the derivatized valency platform molecule and the linker coupling the polynucleotide to the platform molecule for Conjugates 3-I, 3-II, 11-I, 11-II, 11-IV, 11-VI, 11-VIII, 17-I, 17-II, 20-I, 20-II, 20-III, and 20-IV.--

Replacement paragraph for paragraph on page 20 beginning at line 9:

--Conjugation of a biological or ~~chemical~~ synthetic molecule to the chemically-defined platform molecule may be effected in any number of ways, typically involving one or more crosslinking agents and functional groups on the biological or ~~chemical~~ synthetic molecule and valency platform molecule.--

Replacement paragraph for paragraph on page 25 beginning at line 4:

Another procedure involves introducing alkylamino or alkylsulfhydryl moieties into either the 3' or 5' ends of the polynucleotide by appropriate nucleotide chemistry, e.g., ~~phosphoramidate~~ phosphoramidite chemistry. The nucleophilic groups may then be used to react with a large excess of homobifunctional cross-linking reagent, e.g., dimethyl suberimidate, in the case of alkylamine derivatives, or an excess of heterobifunctional cross-linking reagent, e.g., m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) or succinimidyl (4-iodoacetyl) aminobenzoate (SIAB), for the alkylsulfhydryl derivatives. Once excess cross-linker is removed, the polynucleotide derivatives are reacted with amino groups on the platform molecule.

Alternatively, the sulfhydryl group may be reacted with an electrophilic center on the platform, such as a maleimide or α-haloacetyl group or other appropriate Michael acceptor.--

Replacement paragraph for paragraph on page 26 beginning at line 7:

--Immunogens that are involved in ~~antibody- mediated~~ antibody-mediated pathologies may be external (foreign to the individual) immunogens such as allergens, ~~α-sperm~~ sperm associated with male infertility, the rheumatic fever carbohydrate complex, the RBC Rh/D antigen associated with hemolytic disease of the newborn, biological drugs, including native biological substances foreign to the individual such as therapeutic proteins, peptides and antibodies, and the like or self-immunogens (autoimmunogens) such as those associated with thyroiditis (thyroglobulin), stroke (cardiolipin) and myasthenia gravis (acetylcholine receptor).--

Replacement paragraph for paragraph on page 26 beginning at line 19 and continuing through page 27, line 12:

--Analogs to such immunogens may be identified by screening candidate molecules to determine whether they (a) bind specifically to serum antibodies to the immunogen and (b) lack T cell epitopes. Specific binding to serum antibodies may be determined using conventional immunoassays and the presence or absence of T cell epitopes may be determined by conventional T cell activation assays. In this regard, an analog which "binds specifically" to serum antibodies to the immunogen exhibits a reasonable affinity thereto. Further in this regard, it should be recognized that testing for T cell epitopes is conducted on a subject-by-subject basis using T cells taken from an intended recipient or from various patients that represent the target population of recipients. The presence or absence of T cell epitopes may be determined using the tritiated thymidine incorporation assay described in the examples. The presence of T cell ~~eptiopes~~ epitopes can also be determined by measuring secretion of T cell-derived lymphokines by methods well known in the art. Analogs that fail to induce statistically significant incorporation

of thymidine above background are deemed to lack T cell epitopes. It will be appreciated that the quantitative amount of thymidine incorporation may vary with the immunogen. Typically a stimulation index below about 2-3, more usually about 1-2, is indicative of a lack of T cell epitopes.--

Replacement paragraph for paragraph on page 29 beginning at line 1:

--Carbohydrates such as oligosaccharides can be modified to contain a sulfhydryl-containing linker (Wood, S.J. and Wetzel, R., Bioconjugate Chemistry, 3:391 (1992)). The sulfhydryl group is used for conjugation to a haloacetylated platform. Alternatively, carbohydrates can be oxidized to generate aldehydes which ~~is reacted in the presence of $NaCNBH_3$ with amino platforms to form conjugates~~ are reacted with amino platforms in the presence of $NaCNBH_3$ to form conjugates.--

Replacement paragraph for paragraph on page 29 beginning at line 9:

--Lipids such as ~~glycol-lipids~~ glycolipids containing an ethanolamine group are reacted with an activated carboxylate on the platform. Lipopolysaccharides containing sugar units are oxidized to generate aldehydes which are reacted in the presence of $NaCNBH_3$ with amino platforms to form conjugates by reductive amination.--

Replacement paragraph for paragraph on page 30 beginning at line 15:

--The following reaction schemes illustrate methods of synthesizing derivatized chemically-defined valency platform molecules within the present invention. In this example, ~~DMTr=dimethoxytrityl~~ DMTr=4,4'-dimethoxytriphenylmethyl; Tr=trityl; Bz=benzoyl; Cp=deoxycytidine monophosphate, CE=cyanoethyl; CPG=controlled pore glass, DMF = ~~dimethyl formamide~~ dimethylformamide, DCC = dicyclohexylcarbodiimide, TFA = trifluoroacetic acid, CDI = carbonyl diimidazole, Ts = tosyl (para-toluene sulfonyl), DIPAT =

diisopropyl ammonium tetraazolide, ~~TBDMSCl = tetrabutyl~~ TBDMSCl = tertbutyl dimethyl silyl chloride, TBAF = tetrabutyl ammonium fluoride, NMMO = ~~N-methylmorpholine oxide~~ N-methylmorpholine-N-oxide.--

Replacement paragraph for paragraph on page 45 beginning at line 26 continuing through page 46, line 10:

--Compound 4 - [Mono-N-carbobenzyloxy-3,6-dioxa-~~1.8~~ 1,8-diaminooctane]: A solution of 14.3 mL (17.1 g, 100 mmol) of benzylchloroformate in 200 mL of CH_2Cl_2 was added dropwise over a 1 hour period to a solution of 29.0 mL (29.6 g, 200 mmol) of ~~2,2'-(ethylenedioxy)-diethylamine~~ 1,2-bis-(2'-aminoethoxy)ethane (Fluka) in 100 mL of CH_2Cl_2 at 00. The mixture was stirred at room temperature for 24 hours and 1 N HCl was added until the aqueous layer remained acidic (pH less than 2). The aqueous layer was washed with three 50 mL portions of CH_2Cl_2 and neutralized with 1 N NaOH until the pH was above 13. The basic aqueous layer was extracted with five 75 mL portions of CH_2Cl_2. The combined CH_2Cl_2 layers were dried ($MgSO_4$), filtered, and concentrated to yield 12.7 g (45%) of compound 4 as a thick oil: ^{1}H NMR ($CDCl_3$) d 2.82 (bd s, 2H), 3.30-3.60 (m, 12H), 5.10 (s, 2H), 5.75 (bd s, 1H), 7.20-7.40 (m, 5H); ^{13}C NMR ($CDCl_3$) d 41.1, 41.8, 66.5, 70.0, 70.2, 70.4, 73.5, 127.9, 128.0, 128.4, 136.9, 156.4.--

Replacement paragraph for paragraph on page 70 beginning at line 1:

--Compound 45: A mixture of 560 mg (1.4 mmol) of compound 44 and 1.69 g (6.0 mmol) of compound 4 was heated under nitrogen at 150° for 4 hours. The mixture was partitioned between 50 mL of EtOAc and 25 mL of 1N HCl, and the HCl layer was extracted with 25 mL of CH_2Cl_2. Combined EtOAc and CH_2Cl_2 extracts were washed with saturated $NaHCO_3$ solution, dried (K_2CO_3), filtered, and concentrated to a viscous residue. Purification by chromatography on silica gel (gradient 95/5 to 90/10 CH_2Cl_2/MeOH) provided 300 mg (19%) of

compound 45 as a viscous oil: TLC, Rf = 0.24 ~~(90/10 CH_2Cl_2)~~ (90/10 CH_2Cl_2/MeOH); ^{1}H NMR ($CDCl_3$) d 2.40 (t, 6H), 3.38 (s, 6H), 3.39-3.48 (m, 12H), 3.52-3.67 (m, 32H), 5.13 (s, 6H), 5.62 (bd s, 3H) 6.80 (bd s, 3H), 7.40 (s, 15H).

Replacement paragraph for paragraph on page 71 beginning at line 9:

--Compound 47 - S-(6-hydroxyhexyl)isothiuronium chloride: 11.1 g (146 mmol) of thiourea was added to a solution of 16.6 mL (20.0 g, 146 mmol) of 6-chlorohexanol in 49 mL of ethanol and the mixture was refluxed for 24 hours. The mixture was cooled to 0^0 and the product crystallized. The crystals were collected by vacuum filtration and dried to give 28.4 g (92%) of compound 47 as a white solid: mp 122-1240; ^{1}H-NMR (DMSO) 1.40 (m, 4H), 1.65 (m, 2H), 3.21 (t, 2H), 3.41 (t, 2H), 9.27 and 9.33 (overlapping broad singlets, 4H); Anal. Calc'd for ~~$C_7H_{17}ClN_2O_5$~~ $C_7H_{17}ClN_2OS$: C, 39.51; H, 8.06; N, 13.17; S, 15.07. Found: C, 39.69; H, 8.00; N, 13.01; S, 15.16.--

Replacement paragraph for paragraph on page 86 beginning at line 9:

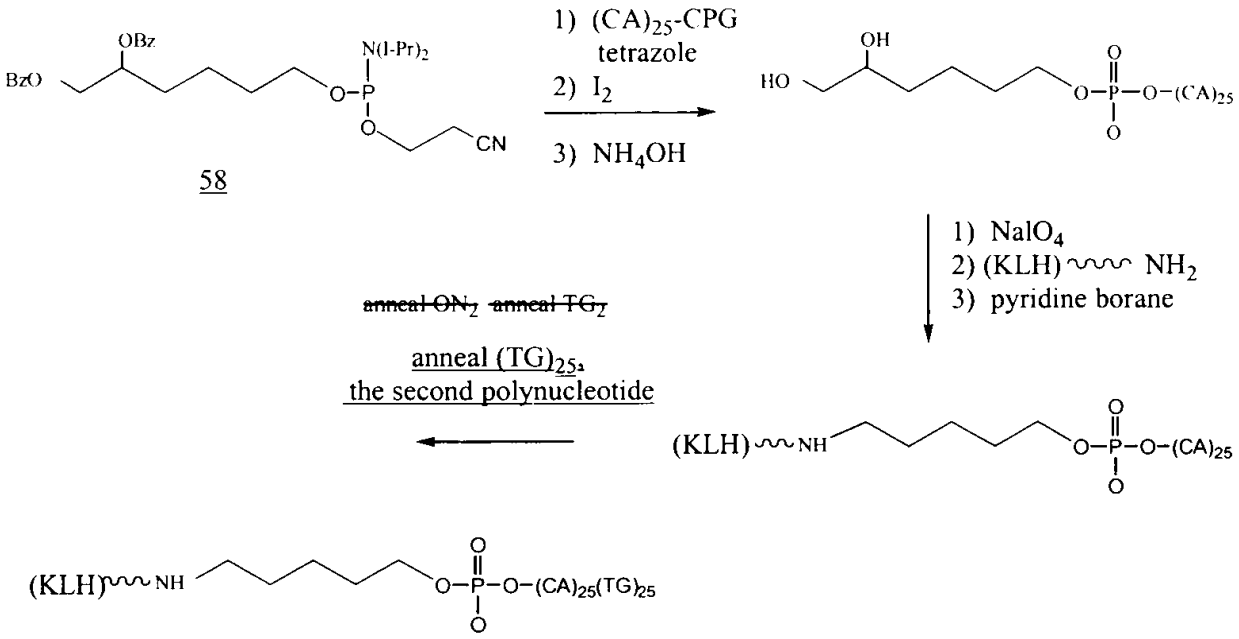
--Compound G - Platform with Four ~~N-Hydroxysuccinimidyl~~ N-Succinimidyl Esters. A solution of 126 mg (0.1 mmol) of F and 46 mg (0.4 mmol) of N-hydroxysuccinimide in 5 mL of anhydrous THF is prepared. The mixture is cooled to 0° and 103 mg (0.5 mmol) of dicyclohexylcarbodiimide is added. The mixture is stirred allowing to come to room temperature over several hours. The solids are removed by filtration, and the filtrate is concentrated to provide G which can be purified by silica gel chromatography.--

Replacement paragraph for paragraph on page 94 beginning at line 20 through page 95, line 18:

The polynucleotide d-[DMTr-(bzCp(CE)bzA)$_{10}$] was prepared on a Milligen 8800 Prep Scale DNA synthesizer (See Figure 6A) following the manufacturer's protocols for DNA

phosphoramidite synthesis. The synthesis was carried out on 10 g of DMTr-d-bzA-CPG support with a nucleoside loading of 30.0 μmol/g. The final DMTr blocking group was removed using the machine protocol. Milligen activator solution, Cat. No. MBS 5040 (45 mL) and 0.385 g of compound 51 (see Reaction Scheme 11) were added to the reaction and the suspension was mixed for 8 minutes by argon ebullition. The mixture was oxidized by the usual machine protocol and the support-bound polynucleotide was collected by filtration, air dried and treated with 100 mL of concentrated ammonia for 16 hours at 55°C. When cool, the mixture was filtered through a Gelman 10 ~~μ~~ μm polypropylene filter. The filter was washed with 200 mL of 2 mM NaCl adjusted to pH 12 with NaOH. The filtrate was then applied to ~~a~~ an Amicon chromatography column (0.45 x 9.4 cm, 150 mL) which had been packed with Q-Sepharose (Pharmacia, Peapack, NJ) equilibrated first with 3M NaCl and then with 2 mM NaCl, pH 12. The column was eluted with 500 mL of a linear gradient (2 mM NaCl, pH 12 to ~~mL~~ 1.3 M NaCl, pH 12), then washed with 1.3 M NaCl, pH 12 until all U.V. absorbing material came off. Fractions which absorbed at 260 nm were further analyzed by polyacrylamide electrophoresis and those containing pure product were pooled. The pool (120 mL) was treated with 240 mL of cold isopropanol and stored for 30 minutes at -20°C. The precipitate was collected by centrifugation in a Sorvall RC 3B centrifuge using a model H-6000A rotor for 15 minutes at 3000 rpm and 4°C to yield DMTr-5'-modified $(CA)_{10}$ (14946 A_{260} units, 498 mg, 62.2 ~~μM~~ μmol, 20% based on 300 ~~μM~~ μmol CPG nucleoside.)--

Replacement entry for reaction scheme on page 98 (single underline indicates insertion):



Replacement paragraph for paragraph on page 99, beginning at line 2:

--Compound 58 was coupled to $(CA)_{25}$ as the final step of automated synthesis which incorporates the elements of an acyclic triol moiety (ACT). Forty-nine sequential steps were carried out using alternating dC and dA phosphoramidites beginning with 10 g of DMT-d-bzA-CPG support with a nucleoside loading of 30 μmol/g. The DMTr blocking group was removed from the resulting ~~d-[DMTr-(bzCp(CE)bzA)$_{25}$]~~ d-[DMTr-(BzCp(CE)BzA)$_{25}$], and 40 mL of activator solution (Milligen, Cat. No. MBS 5040) and 800 mg of compound 58 were added to the reaction mixture. The suspension was mixed for 8 minutes by argon ebullition and subjected to a conventional oxidation step. The support bound polynucleotide was removed from the reaction vessel, air dried, and treated with 100 mL of concentrated ammonia for 40 hours at 55° C. When cool, the mixture was filtered through a Gelman 10 μm polypropylene filter and the filtrate was then purified by conventional ion exchange chromatography. Fractions which absorbed at 260 nm were further analyzed by polyacrylamide gel electrophoresis and those containing pure product were combined and precipitated with isopropanol to provide 510 mg (31.9 μmol, 10%) of the ACT-modified $(CA)_{25}$.--

Replacement paragraph for paragraph on page 99, beginning at line 25:

--To a solution of 100 mg (2.5 Tmol) of $NaIO_4$-treated ACT-modified $(CA)_{25}$ in 1.33 mL of 50 mM sodium borate pH 8.0 was added 31.3 mg (0.208 μmol) of KLH and 2.0 mg (31.8 μmol) of pyridine borane ~~$NaCNBH_3$~~. The mixture was kept at 37°C for 72 h, and the product was purified by chromatography on S-200.--

Replacement paragraph for paragraph on page 100 beginning at line 3:

--The equivalent amount of $(TG)_{25}$ was added to the single-stranded PN-KLH conjugate and the tube was heated at 900C for ten minutes and then allowed to cool to room temperature over an hour and a half. Precipitation with isopropyl alcohol yielded 53 mg of ~~18~~ product, PN-KLH; Tm (0.15 M NaCl, 0.01 M sodium citrate, pH 6.8) 73.40, 31.1% hyperchromicity; 98% double stranded as determined by HPLC comparison to standards consisting of sample annealed with excess $(TG)_{10}$, unannealed conjugate, and unannealed $(TG)_{10}$ (Shodex Protein KW 8025 column, 0.05 M NaH_2PO_4, pH 6.5, 0.5 M NaCl). This conjugate may be represented by the formula

$KLH\text{-}[NH(CH_2)_5OPO_2,O\text{-}(CA)_{25}:(TG)_{25}]_{\sim 5}$

(assuming a molecular weight of 10^5 for KLH) and is designated "PN-KLH."--

Replacement paragraph for paragraph on page 100, beginning at line 22:

--Conjugate 3-II was tested for its ability to tolerize mice that had been immunized with an immunogenic form of the polynucleotide, PN-KLH.

Replacement caption for the caption on page 103, beginning at line 18:

--Conjugation of Tr-5' Modified $(CA)_{10}$ to Valency Platform Molecule 20 - Preparation of Single-Stranded Conjugate 20-I--

Replacement paragraph for paragraph on page 103, beginning at line 21, continuing through page 104, line 29:

969 μL (789 mg, 3.89 mmol) of tri-n-butylphosphine was added to a solution of 918 mg (0.14 mmol) of Tr-5'-modified $(CA)_{10}$ in 30 mL of H_2O under argon atmosphere. The mixture was stirred for 1 hour and then 2.4 mL of a 3M NaCl solution was added followed by 42 mL of isopropanol which had been sparged with helium to remove oxygen. The mixture was placed in a freezer at -20°C for 1 hour and then centrifuged at 3000 rpm for 30 minutes. The supernatant was removed and the oily residue was dissolved in 15.5 mL of helium sparged H_2O. 1.24 mL of 3M NaCl and 21.7 mL of helium sparged isopropanol was added to the mixture. The resulting mixture was then placed in a freezer at -20°C for 1 hour and centrifuged at 3000 rpm for 20 minutes. The oily pellet was dried under vacuum for 18 hours to yield a solid. The solid was dissolved in 6 mL of helium sparged H_2O to give a total volume of 6.4 mL. The amount of DNA was 863 mg as determined by UV absorbance at 260 nm (0.033 mg per unit of absorbance in pH 7.5 phosphate buffered saline). The solution was transferred to a 50 mL three-neck flask under argon. One neck of the flask had an argon gas inlet while the other two necks were stoppered. The total volume was adjusted to 7.7 mL with H_2O and 0.87 mL of helium sparged 1M sodium phosphate buffer, pH 7.8, and 0.97 mL of MeOH. 1.9 mL (33.63 mg, 0.025 mmol) of a 17.7 mg/mL solution of compound 20 in MeOH was added to the mixture. The resulting mixture was stirred under argon for 20 hours and then diluted to 100 mL with a solution comprising 0.1 M NaCl, 0.05 M sodium phosphate, pH 7.5, and 10% MeOH. Purification was accomplished by chromatography on Fractogel® (equilibration: 0.1 M NaCl, 0.05 M sodium phosphate, pH 7.5, 10% MeOH: elution gradient 0.5 M NaCl, 0.05 M sodium phosphate, pH 7.5, 10% MeOH to 0.8 NaCl, 0.05 M sodium phosphate, pH 7.5, 10% MeOH). Fractions containing pure conjugate 20-I as evidenced by HPLC and polyacrylamide gel electrophoresis were collected in 232 mL of eluent. The product and salts were precipitated by adding an equal volume of isopropanol and

placing same in a freezer at -20°C for 1 hour. Dialysis against H_2O (2 x 100 vol) gave 335 mg of conjugate 20-I (32 mL of 10.47 mg/mL, 0.033 mg/absorbance unit at 260 nm, assumed).--

Replacement caption for caption on page 105, beginning at line 20:

--Alternative Conjugation of TR-5'-Modified $(CA)_{10-20}$, Preparation of Single Stranded Conjugate 20-I--

Replacement paragraph for paragraph on page 105, beginning at line 22 continuing through page 106, line15:

--10 equivalents of tri-n-butylphosphine are added to a 10 mg/mL solution of Tr-5'-modified $(CA)_{10}$ in He sparged with 100 mM pH 5 sodium acetate. The mixture is stirred for 1 hour and then precipitated with 1.4 volumes of isopropyl alcohol (IPA). The mixture is placed in the freezer at -20°C for 1 hour and centrifuged at 3000 rpm for 20 minutes. The supernatant is removed and the pellet is dissolved to 10 mg/mL in He sparged IPA. The mixture is placed in the freezer at -20°C for 1 hour and centrifuged at 3000 rpm for 20 minutes. The pellet is dried under vacuum for 18 hours to give a solid. A 50 mg/mL solution of the solid is prepared in He sparged 100 mM pH 10 sodium borate buffer. 0.25 equivalents of compound 20 as a 40 mg/mL solution in 9/1 MeOH/H_2O is added to the mixture. The mixture is stirred at room temperature for 3-20 hours and diluted (0.1 M NaCl, 0.05 sodium phosphate, pH 7.5, 10% MeOH). Purification is accomplished by chromatography on ~~Factogel~~ Fractogel® (equilibration; 0.1 M NaCl, 0.05 M sodium phosphate, pH 7.5, 10% MeOH: elution gradient; 0.5 M NaCl, 0.05 M sodium phosphate, pH 7.5, 10% MeOH to 0.8 M NaCl, 0.05 sodium phosphate, pH 7.5, 10% MeOH). Fractions containing pure 20-I, as evidenced by HPLC and polyacrylamide gel electrophoresis, were collected. The product and salts are precipitated by adding an equal volume of IPA and standing in the freezer at -20°C for 1 hour. Dialysis against H_2O (2 X 10 vol) give 20-I.--

Replacement caption for the caption on page 106, beginning at line 23:

--Second Alternative Conjugation of Tr-5'-Modified $(CA)_{10-20}$. Preparation of Single Stranded Conjugate 20-I--

Replacement paragraph for the paragraph on page 106, beginning at line 25, continuing through page 107, line 15:

--4.8 mL of tri-n-butylphosphine was added to a solution of 7.75 g of Tr-5'-modified $(CA)_{10}$ in 104 mL of Ar sparged 100 mM pH 5 sodium acetate under N_2. The mixture was stirred for 1 hour and then precipitated with 232.5 mL of IPA. The mixture was placed in a freezer for -20°C for 1.5 hours, centrifuged at 3000 rpm for 20 minutes and then frozen at -20°C for 24 hours. The supernatant was removed and the pellet was dissolved in 170 mL He sparged 0.3 M NaCl solution. The mixture was again precipitated with 232 mL of Ar sparged IPA. The mixture was then placed in a freezer at -20°C for 2 hours, centrifuged at 3000 rpm for 20 minutes and then ~~from~~ frozen at -20°C for 11 hours. The supernatant was decanted and the pellet was dried under vacuum for 12 hours to give a solid. A solution of the solid was prepared in 110 mL of Ar sparged 100 mM pH 10 sodium borate buffer. 406 mg of compound 20 as a solution in 4.4 mL of 9/1 MeOH/H_2O was added to the mixture. The mixture was stirred at room temperature for 2 hours. The product mixture contained 62% of 20-I by high-pressure ion chromatography, Waters Gen Pak Fax column (100 X 4 mm), 60°C, linear gradient from 65%A/35%B to 18%A/82%B; A=0.05 M NaH_2PO_4, pH 7.5, 1 mM EDTA, 10% MeOH (v/v); B=0.05 M NaH_2PO_4, pH 7.5, 1 M NaCl, 1 mM EDTA, 10% MeOH (v/v), eluting at 19.5 minutes.--

Replacement paragraph for paragraph on page 114 beginning at line 17 continuing through page 115 line 19:

--A serum sample taken from each mouse was assessed for the presence of anti-DNA antibody by ELISA. Falcon Probind 96 well microtitration assay plates (Becton Dickerson,

Oxnard, CA) were coated with 100 µL/well of $(PN)_{50}$-D-EK (a co-polymer of D-glutamic acid and D-lysine) at a concentration of 50 µg/mL overnight at 40C. The plates were washed twice with PBS without calcium and magnesium and 0.05% Tween 20 (wash buffer) using a M96V plate washer (ICN Biomedical, Inc., Irvine, CA). Plates were blocked for 1 hour at room temperature in PBS containing 1% gelatin (Norland Products, Inc., New Brunswick, NJ) and 0.05% Tween 20. Plates were washed twice with wash buffer before the addition of serum samples or standards. Serum samples and standards were prepared in a diluent containing PBS with 1% gelatin, 0.05% Tween 20 and 10% goat serum. Plates were incubated with serum samples for 60 to 90 minutes at 37°C and then the wells were washed four times with wash buffer. Biotinylated goat anti-mouse IgG (Sigma Chemical Co., St. Louis, MO) was diluted 1/1000 in blocking solution containing 10% goat serum. The plates were incubated for 1 hour at 37°C and washed four times. The substrate, OPD (Sigma Chemical Co., St. Louis, MO),was added. The plates were incubated in the dark until the highest reading of the highest standard was approximately 1 OD unit by an ELISA plate reader at OD 450 nm (Bio-Tek Instruments, Winooski, VT). The reaction was stopped with 50 µL of 3M HCl and the plates were read at 490 nm. The reference positive serum was included in each microtitration plate and the positive wells from each assay were within the sensitivity range of the reference curve 95% of the time. In the later bleeds, some positive samples exceeded the reference curve. However the most dilute mouse serum sample was within the range of the reference curve. No significant binding was observed by normal control negative serum. The results are shown in Figure 15.--

Replacement paragraph for paragraph on page 117 beginning on line 17:

~~--Melittin Peptide #8.~~

~~H_2N-Cys-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln-Gly-CO_2H (SEQ. ID NO.: 9).--~~

Replacement paragraph for paragraph on page 117 beginning on line 20:

~~--Melittin Peptide #9.~~ Melittin Peptide #8.

$(H_2N$-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln$)_2$-Lys-Cys-Gly-CO_2H (SEQ. ID NO.: 10).--

Replacement entry for the entry on page 119, first line Table 7:

--~~Table 7~~ Table 6--

Replacement entry for the entry on page 119, Table 7, column entitled "Valence platform", first entry (for conjugate number 1):

--~~5~~ 60--

Replacement entry for the entry on page 119, Table 7, column entitled "Peptide conjugated", last entry:

--~~8~~ 9--

Replacement paragraph for the paragraph on page 122, beginning at line 14:

--T Cells from mice primed with melittin showed T cell proliferation in response to the whole melittin molecule and to C-terminal melittin peptides 3, 4, and 5 (Figure 8). However, C-terminal peptides 1 and 2 induced no significant T cell proliferation. Melittin peptides 2 and 5 were conjugated to PEG. Like melittin peptide 2, the PEG conjugate of melittin peptide 2 ~~(Conjugate 2)~~ also did not induce significant T cell proliferation.--

Replacement paragraph for the paragraph on page 122, beginning at line 25 continuing through page 123, line 12:

--Mice treated with ~~Conjugate 2~~ the conjugate prepared as described above (10 mg/kg, 200 μg/mouse), had significantly lower levels of anti-melittin peptide 2 antibodies (Figure 9) and also lower levels of anti-melittin antibodies (Figure 10) as compared to the control Balb/c mice treated with formulation buffer. Spleen cells from mice treated with buffer control or ~~Conjugate 2~~ the conjugate were assayed for the ability of antibody- forming cells to produce anti-melittin or anti-melittin peptide 2 antibodies as measured in a soluble ELISA assay. As shown in Figure 11,

the levels of anti-melittin peptide 2 antibody forming cells in the ~~Conjugate 2~~ conjugate treatment group were significantly lower than in the control group which was administered formulation buffer. Mice treated with Conjugate 4, a conjugate of peptide 5 which contains a T cell epitope, failed to reduce the titer of antibodies to peptide 5 in treated mice. Thus, the conjugate containing a T cell epitope was not a tolerogen (Figure 12). In fact, rather than reduce the response, the levels of anti-peptide antibody may have increased slightly.--

Replacement paragraph for the paragraph on page 125 beginning at line 12:

--Two different tolerogens were constructed to determine if the orientation of the peptide on the PEG conjugate affects its ability to induce tolerance. The peptide was covalently bound to valency platform molecule 3 through its C-terminal end to make melittin conjugate 3. Groups (3/group) of mice primed with melittin were treated, i.p., with conjugates or with saline. Five days later all of the mice, including the non-treated control group, were boosted with 5 μp of melittin. Six days later the mice were sacrificed, their spleens were harvested and the number of peptide specific pfc determined. As illustrated in Table ~~6~~ 8, both orientations were effective in reducing the number of peptide-specific pfc/10^6 spleen cells in mice primed and boosted with the parent protein Melittin.--

In the Figures:

Please amend Figure 13 as follows:

Under first formula, delete "~~Conjugate #1, R = "H_2N-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln-Lys-Cys-Gly-CO_2H.~~" and replace with --Conjugate #1, R = "H_2N-Cys-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln-Gly-CO_2H.--